Muscle development: **A myogenic switch**

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Experiments manipulating the level of the basic helix–loop–helix transcription factor Twist in the *Drosophila* embryo have revealed a novel role for this protein in a 'myogenic switch' during early development.

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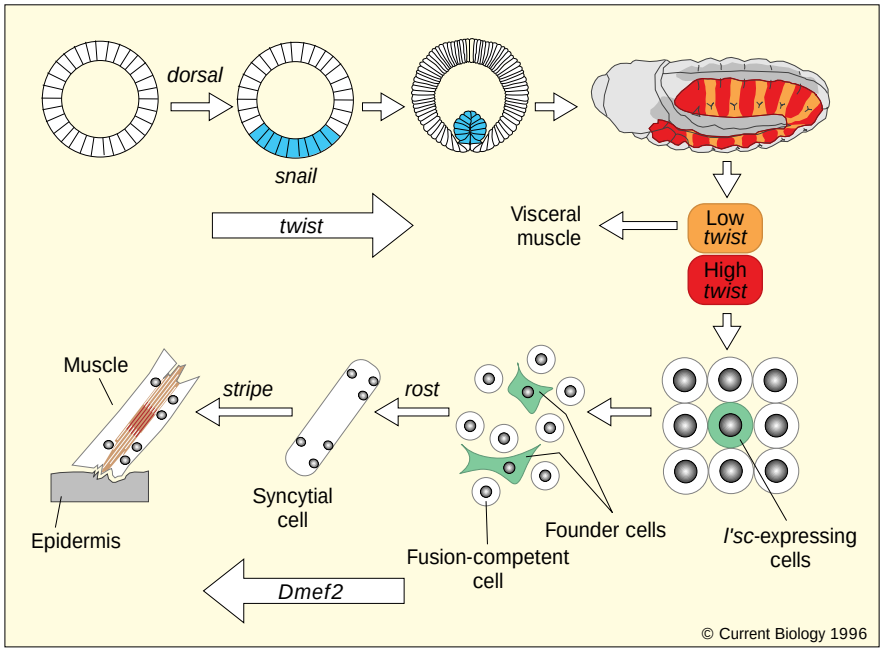
The somatic, or body wall, musculature of the *Drosophila* embryo comprises a pattern of thirty distinct muscles in each hemisegment. A framework for understanding the development of these muscles has recently emerged (Fig. 1). Briefly, muscle progenitors are selected from a localized domain of the mesoderm by a mechanism similar to the well-documented singling out of neuroblasts in the ectoderm. This process involves lateral inhibition mediated by the neurogenic genes, and is revealed by the progressively restricted expression in the somatic mesoderm of the 'proneural' gene *lethal of scute* (*l'sc*) [1]. The muscle progenitors produce a small number of 'founder cells', one for each muscle, which seed the final muscle pattern.

In mutant embryos where myoblast fusion does not occur [2], the founder cells are revealed as a distinct population of myoblasts, which in normal development would fuse with so-called fusion-competent myoblasts to form the final syncytial muscles, which then attach to particular sites on the epidermis. Genes that mark, or are required for, many of these steps have been identified and include *S59* and *apterous*, which are founder cell markers [3], and *Dmef2*, *rost* and *stripe*, which are required for differentiation, cell fusion and muscle attachment, respectively [4–6] (Fig. 1). However, rather little is known of the earliest steps. For example, what are the genetic mechanisms that operate in the subdivision of the mesoderm to produce the domain from which the muscle progenitors derive?

The mesoderm is defined during pre-gastrulation development in response to a gradient of nuclear Dorsal protein, and is revealed by the expression domain of genes like *snail* in the ventral region of the cellular blastoderm [3] (Fig. 1). These cells invaginate at gastrulation to form the mesoderm proper, but at this stage they are not committed to a specific cell fate [3]. The commitment to make somatic muscle, and indeed the other mesodermal derivatives including the visceral (or gut) musculature and the heart, occurs in the next few hours. Until recently, there was no information on the genes required for the commitment to

Figure 1



An outline of *Drosophila* somatic muscle development. Mesoderm (blue) is defined in response to *dorsal* (*dl*) and is revealed in the cellular blastoderm by the expression of *snail* in ventral cells, which invaginate to form the mesoderm proper. The *twist* gene is required for mesoderm formation, and is subsequently expressed at high (red) and low (orange) levels in segmentally repeated domains. The low-level domain gives rise to visceral muscle. Somatic muscle progenitors expressing *lethal of scute* (*l'sc*) are singled out from the high *twist* domain and produce founder cells, revealed by markers such as *S59* and *apterous*. *Dmef2* is then required for the later steps of differentiation. In a process requiring genes like *rolling stone* (*rost*), founders fuse with other myoblasts (also derived from the high *twist* domain) to form syncytial muscles, which then attach to specific epidermal sites under the influence of genes like *stripe*.

the somatic muscle lineage. But a recent paper from Baylies and Bate [7], which reports a powerful combination of overexpression and underexpression experiments, provides compelling evidence that the gene *twist* plays a key role.

Twist is a basic helix–loop–helix protein expressed in response to Dorsal, and is required for gastrulation and hence for formation of the mesoderm [3,8]. However, *twist* continues to be widely expressed in the mesoderm as mesodermal cells enter specific differentiation pathways. It is not, however, expressed uniformly, and in each embryonic segment there is a domain of cells where levels of Twist are high, and a domain where they are relatively low (Fig. 1). The analysis of embryos carrying a mesodermal cell marker has shown that the high Twist domain gives rise to somatic muscle, and that visceral muscle derives from the low Twist domain [9]. Although the function of *twist* in gastrulation and mesoderm formation has been known for many years [8], it was the study of the possible role of *twist* after gastrulation that revealed its role as a 'myogenic switch'.

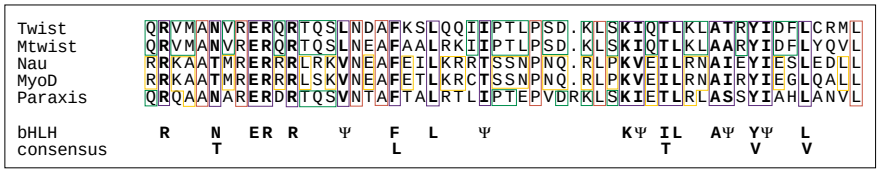
Baylies and Bate [7] used the GAL4–UAS system to generate flies in which Twist was expressed throughout the post-gastrulation mesoderm; they placed the *twist* gene under the control of the upstream activating sequence (UAS) that responds to the yeast transcriptional activator GAL4, and used an appropriate enhancer to confer the desired expression pattern on GAL4. In these flies, the domains that would normally express low levels of Twist now expressed higher levels. They found that, despite some minor defects, the somatic muscles were essentially normal. In contrast, there were fewer visceral muscle progenitors, and heart cells were missing or deranged. Most provocatively, ectopic, somatic muscle-like syncytia were induced where the visceral musculature and heart normally develop. These findings suggest that high levels of Twist inhibit some differentiation pathways and direct cells down the route that makes somatic muscle. In this model, lower levels of Twist allow cells to differentiate along other pathways, like visceral muscle and heart. It is not yet known whether this lower amount of Twist is actually required for these pathways.

It was obviously important to ask what happens when Twist expression is reduced to below wild-type levels. To circumvent the problem of the requirement for *twist* in order to gastrulate, Baylies and Bate [7] exploited an elegant genetic 'trick' and used embryos carrying a temperature-sensitive combination of *twist* alleles. They allowed these embryos to develop normally until gastrulation and then moved them to the restrictive temperature to determine the effect of reducing *twist* activity. The results fitted the model developed from the overexpression studies. They found that the visceral musculature and heart developed essentially normally, but now, and as anticipated, somatic muscle differentiation was grossly defective, although it appears that some muscle differentiation did occur (this may reflect a pathway that does not require high Twist).

This result is reminiscent of the dramatic results obtained in vertebrates during the past decade with other basic helix–loop–helix proteins, namely the MyoD family of muscle transcription factors. They can convert a variety of cultured and embryonic cells, including those of non-mesodermal origin, into skeletal muscle [10,11]. A burning question in this new *twist* study was therefore what would be the effect of ectopic expression of Twist in another germ layer. Strikingly, it was found that, in the ectoderm, ectopic Twist resulted not only in the switching off of epidermal and nervous system differentiation, but also in the production of muscle myosin expressing bi- or tri-nucleate syncytia in the place of epidermis [7]. So, *twist* not only can push mesodermal cells down the somatic muscle differentiation pathway, but it also has a similar effect on non-mesodermal cells. This is why it has been called a 'myogenic switch'.

How can these findings be related to the function of basic helix–loop–helix proteins in other animals in muscle and, more generally, in mesodermal development? The role of *twist* as a myogenic switch recalls the position of the MyoD family at a nodal point in vertebrate skeletal myogenesis [10]. But in *Drosophila* it is *nautilus*, not *twist*, that encodes the protein most closely related in sequence to the MyoD family (Fig. 2). Nautilus expression, however, is restricted to a subset of myoblasts and so it cannot play a general role

Figure 2



The basic helix–loop–helix (bHLH) domains of five proteins implicated in muscle development. Twist and Nautilus (Nau) are from *Drosophila*; Mtwist, MyoD and Paraxis are from mouse. The coloured boxes refer to the following: purple, amino acids matching the bHLH consensus (also highlighted in bold type); red, amino acids shared by all five proteins; green, residues shared by Twist and Mtwist (and sometimes also Paraxis); yellow, amino acids shared by Nau and MyoD (and sometimes also Paraxis). The original consensus sequence for the bHLH domain is from [16]. Accession numbers for the sequences shown are: Twist, X14569; Mtwist, M63650; Nau, X56161; MyoD, M18779; Paraxis, U18658.

in myogenesis. Other animals have proteins with sequences similar to Twist (Fig. 2), but again the function of these proteins appears distinct. For example, the expression pattern of Mtwist in mice suggests a role in mesoderm patterning, but not in promoting skeletal muscle differentiation. Indeed, work in cell culture revealed that Mtwist actually inhibits myogenesis [12] and, more recently, that Mtwist inhibits the function of MyoD and MEF2 in activating reporter gene expression [13].

Currently, the vertebrate basic helix–loop–helix protein with the most similar expression pattern to *twist* is *paraxis* (Fig. 2), but its function remains to be defined. Taken together, this body of work suggests that, although many organisms use basic helix–loop–helix proteins for mesodermal patterning and differentiation, the specific role of the most closely related proteins in different species may be distinct. Nevertheless, one should remember that the picture of the genes involved, and of their role(s), is still very incomplete. The fact remains that the general similarity of mesoderm development and myogenesis in *Drosophila* and various vertebrate species suggests that findings in one organism will continue to advance understanding in others.

From the work in *Drosophila*, it is clear that Twist plays at least two roles during development: first at gastrulation, and second in the subdivision of the mesoderm. How does it function? Twist has generally been thought of as a transcriptional activator of mesodermal genes and, in some cases at least, this appears to be a direct effect [4,14,15]. However, relatively few targets are known and the crucial targets activated when Twist pushes cells down the somatic muscle differentiation pathway remain to be defined. Although *Dmef2* is both a downstream target of Twist and required to make somatic muscle [4], available results do not support the view that the effect of Twist is simply a case of just activating *Dmef2*.

Another possible action of Twist is suggested by recent work showing that it can inhibit transcription in cell culture [13], although it must be stressed that it is not known whether Twist can function like this in the *Drosophila* embryo. In the experiments of Baylies and Bate [7], Twist certainly does downregulate differentiation of many cell types (visceral muscle, heart, epidermis and nerve), which is compatible with such a function. An alternative mechanism that can operate, and may do in these situations, is that Twist activates *snail*, or genes like it, which in turn repress nonmesodermal genes [14]. In the context of transcription, one can readily imagine Twist having different functions — activation or repression, at gastrulation and in myogenesis — depending on its interacting proteins.

This recent body of work on *Drosophila* myogenesis brings into focus three important questions about events both downstream and upstream of *twist*. What is the mechanism by which *twist* promotes somatic muscle differentiation? What are the steps in selecting the muscle progenitor cells? And finally, how is the modulation of *twist* expression along the anterior/posterior axis controlled to produce domains of high and low levels of Twist? In answer to the last question, roles for pair-rule genes and signals from the overlying ectoderm are suggested [7]. Muscle and patterning devotees (and many interested observers) await the results of testing these ideas with interest.

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